LRH: M. N. Krosch et al.

RRH: Population structure of two chironomid species

Comparison of intraspecific genetic structure among related chironomids (Diptera) from New Zealand and Patagonia: disparity between potential and realized dispersal

Matt N. Krosch[1], **Andrew M. Baker**[2], **AND Peter B. Mather**[3]

School of Earth, Environmental and Biological Sciences, Queensland University of Technology, Brisbane, Australia, 4001

Peter S. Cranston[4]

Research School of Biology, Australian National University, Canberra, Australia, 0200

[1] E-mail addresses: m.krosch@qut.edu.au

[2]

[3]

[4]

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Abstract. Population genetic studies of freshwater invertebrate taxa in New Zealand and South America are currently few despite the geologically and climatically dynamic histories of these regions. The focus of our study was a comparison of the influence on realized dispersal in 2 closely related nonbiting midges (Chironomidae) of population fragmentation on these separated austral land masses. We used a 734-base pair (bp) fragment of cytochrome *c* oxidase subunit I (COI) to investigate intraspecific genetic structure in *Naonella forsythi* Boothroyd in New Zealand and *Ferringtonia patagonica* Edwards in Patagonia. We proposed hypotheses about their potential dispersal and, hence, expected patterns of genetic structure in these 2 species based on published patterns for the closely related Australian taxon *Echinocladius martini* Cranston. Genetic structure revealed for both *N. forsythi* and *F. patagonica* was characterized by several highly divergent (2.0–10.5%) lineages of late Miocene–Pliocene age within each taxon that were not geographically localized. Many were distributed widely. This pattern differed greatly from population structure in *E. martini*, which was typified by much greater endemicity of divergent genetic lineages. Nevertheless, diversification of lineages in all 3 taxa appeared to be temporally congruent with the onset of late Miocene glaciations in the southern hemisphere that may have driven fragmentation of suitable habitat, promoting isolation of populations and divergence in allopatry. We argue that differences in realized dispersal post-isolation may be the result of differing availability of suitable habitat in interglacial periods.

Key words: Plio–Pleistocene, phylogeography, *Naonella forsythi*, *Ferringtonia patagonica*, population fragmentation, Gondwana.

Investigating the role of population fragmentation and isolation in driving diversification is crucial for the development of a more holistic understanding of organismal evolutionary history and is a founding principle of modern ecology and phylogeography (MacArthur and Wilson 1967, Avise et al. 1987). Isolation of previously continuous populations in small refugia intersected by unsuitable habitat can interrupt natural dispersal and gene flow in many taxa, potentially leading populations to evolve along separate and sometimes very different trajectories. This process is considered an important factor that globally influences the evolution and maintenance of biodiversity and endemism (Moritz et al. 2000).

Globally, one of the better-studied taxonomic groups is freshwater invertebrates, for which several scenarios of population fragmentation have been tested (e.g., Carson 1983, Hughes et al. 1999, Kelly et al. 2001, Arrivillaga et al. 2002, Ribera and Vogler 2004, Smith et al. 2006a, 2006b, Finn et al. 2007, and see Monaghan et al. 2010). Freshwater invertebrates, particularly those that inhabit lotic systems, often are susceptible to population fragmentation and isolation leading to divergence of genetic lineages in allopatry (Bohonak 1999, Hughes et al. 2008) although certain highly vagile taxa appear capable of mitigating the effects of habitat fragmentation via long-distance dispersal (Hughes et al. 1998, 2000, Berendonk and Spitze 2006). An apparent disparity often exists between the potential dispersal of a taxon, as predicted by physiological traits, and realized dispersal, as inferred by estimates of relative gene flow among populations. Recently, this disparity between potential and realized dispersal in aquatic invertebrates has been addressed among closely allied species that inhabit different regions of the globe (Cook et al. 2012)

Nonbiting midges of the family Chironomidae (Diptera) are ubiquitous in freshwater ecosystems globally and historically have been considered highly dispersive (Oliver 1971,

Armitage et al. 1995). Population genetic studies of chironomids have revealed patterns of small-scale, generally downstream gene flow and isolation by distance effects in North American taxa (Hilburn 1980, Werle 2005), and minimal gene flow among populations of Western Australian freshwater seep-restricted taxa (Martin et al. 2002). Patterns revealed for arguably the best-studied taxon, *Echinocladius martini* Cranston from eastern Australia, suggest that preconceptions of high vagility and genetic homogeneity among populations (i.e., aerial plankton) were quite unfounded (Krosch et al. 2009, Krosch 2011). Several highly divergent (2.8–12.3%) genetic lineages were revealed that were geographically restricted to previously postulated habitat refugia. Even within a single lineage, significant genetic structure was observed among streams within a single catchment and dispersal was largely confined to downstream larval drift (Krosch et al. 2011a), a pattern suggesting that realized dispersal in *E. martini* is quite low. Taken together, these data dramatically alter our understanding of chironomid population dynamics and necessitate testing similar hypotheses of potential vs realized dispersal in other closely related orthocladiine taxa.

Two such taxa, *Naonella forsythi* Boothroyd and *Ferringtonia patagonica* Edwards, inhabit pristine freshwater streams in New Zealand and southern South America, respectively (Boothroyd 1994, Sæther and Andersen 2010). *Naonella forsythi* is known from upland streams and rivers on both major New Zealand islands (Boothroyd 1994). The recently revised taxon *F. patagonica* has been recorded from montane streams in southern Chile and Argentina (Sæther and Andersen 2010). Both taxa are considered to be ecological analogues of *E. martini*, in that they share similar highly constrained habitat preferences and life-history traits and occupy similar ecological niches (Boothroyd 1994, Cranston 2000, Sæther and Andersen 2010). As larvae, all 3 taxa are detritivores, inhabit leaf packs in riffle sections of cold, predominantly

upland, low-order streams, and feed on passing organic matter in the benthos. All are considered multivoltine, and dispersal of winged adults is thought to be predominantly by gravid females searching for sites for oviposition. Close phylogenetic relationships between these species have been established (Krosch et al. 2011b), and thus, the species are good models for testing hypotheses about their potential (as predicted based on patterns in related *E. martini*) and realized dispersal.

In New Zealand, orogeny of the New Zealand Southern Alps caused by convergence of the Australian and Pacific continental plates along the Alpine Fault over the past 6×10^6 y (Ma) (Mercer 1983, Walcott 1998) fragmented populations of many taxa on the South Island and led to divergence of biotic provinces to the northwest (Northern South Island Province [NSIP]; Leathwick et al. 2007) and southeast (Southern South Island Province [SSIP]; Heads 1998, Trewick and Wallis 2001, Wallis and Trewick 2009). Within each province, Plio–Pleistocene glaciation events, including one during the late Pleistocene that is reported to have covered most of the Southern Alps, would have further fragmented populations of some species (Mercer 1983, Suggate and Almond 2005). Indeed, many taxa show patterns of high intraspecific genetic divergence and co-occurrence of divergent genetic lineages of Plio–Pleistocene age in sympatry (Wallis and Trewick 2009, Pons et al. 2011). Only limited data are available for freshwater taxa, and none for macroinvertebrates, but some fish species exhibit marked genetic divergence among major river catchments and evidence exists for potential river-capture events in the NSIP (Waters and Wallis 2000, Smith et al. 2005, Burridge et al. 2008). Many river catchments in the NSIP are quite large (several hundred kilometres long) and the upper reaches of many are thought to have been affected by Plio–Pleistocene glaciations. Taken together, the NSIP is an ideal region in which to test patterns of relationships among populations of freshwater taxa and

represents the focal point of our sampling of *N. forsythi*.

In western South America, subduction of the Nazca plate under the South American plate apparently drove tectonic uplift, initiated most recently in the late Pliocene, giving rise to the Andes mountain range (Lagabrielle et al. 2004). In addition, since the initiation of tectonic uplift, several glaciation events covered much of the southern Andes from the Pacific coast to the Argentine plains (Mercer 1983, Rabassa and Clapperton 1990, Ehlers and Gibbard 2007, Rabassa et al. 2011). The climatic and geological history of southern South America appears to have influenced population structure in endemic taxa, but available data are limited. Populations of some widespread taxa show isolation and marked genetic structure across the mountain range congruent with bisection by Andean uplift (Marchelli and Gallo 2006, Ruzzante et al. 2006, Ramirez et al. 2008, Gonzalez-Ittig et al. 2010). The genetic signal of population bisection across the Andes in other taxa appears to have been masked by subsequent, or perhaps coincident, historical glaciation events followed by subsequent postglacial range expansions resulting in sympatry among divergent genetic lineages (Close et al. 1978, Kim et al. 1998, Allnutt et al. 1999, Palma et al. 2002, Marchelli and Gallo 2004, Muellner et al. 2005). Genetic data for freshwater organisms, particularly macroinvertebrates, from Andean streams are extremely limited, but taxa that have been studied show patterns of historical population isolation and high intraspecific divergence (Foighil et al. 1999, Ruzzante et al. 2006, Zemlak et al. 2008, 2011, Sabando et al. 2011).

Our goals were first to elucidate patterns of intraspecific genetic structure in *N. forsythi* from New Zealand and *F. patagonica* from southern South America and to compare these with published patterns for other taxa from those regions. We used population genetic analyses and gene trees to test hypotheses concerning population fragmentation and expansion, divergence

times, gene flow, and dispersal. Subsequently, we considered these data in terms of the biogeographical processes that may have influenced evolution in *N. forsythi* and *F. patagonica*. Specifically, for *N. forsythia*, we predicted divergence would be greatest among streams within large river catchments, such that tributaries may represent distinct populations that do not disperse regularly among streams. For *F. patagonica*, we predicted that genetic divergence would be greatest among populations separated by the Andes mountain range.

Second, we considered patterns of genetic structure in *N. forsythi* and *F. patagonica* in terms of potential vs realized dispersal, in light of published patterns for closely related, ecologically analogous *E. martini* from Australia. Based on patterns in *E. martini*, we formed predictions about the potential dispersal of *N. forsythi* and *F. patagonica*. Thus, we expected a pattern of several highly divergent, geographically localized mitochondrial lineages within each taxon. We acknowledge that the underlying causes of habitat fragmentation differed in detail in each region, but our goal was to assess whether our study taxa might have responded to such events in similar ways. Therefore, a null hypothesis for our study is that patterns of genetic structure are influenced by factors other than biogeographical events peculiar to each region.

Methods

Study sites

Naonella forsythia.—We collected larvae and pupae of *N. forsythi* from 6 sites on the South Island of New Zealand (Fig. 1B). Sampling sites were mostly in the Nelson Lakes region, with 1 site on the Lewis Pass (site 6). All 6 sites were on tributaries in the Buller River catchment. Inclusion of sites from additional catchments would have been beneficial, but the Buller River is a major drainage in the northwest South Island (~170 km long) and the upper

158 reaches were affected by glaciations during the Pliocene (Suggate 1990). Given that significant
159 genetic structure was reported within a single catchment for *E. martini*, but also that multiple
160 divergent lineages were sampled from some catchments, we considered that this sampling design
161 could represent genetic structure adequately within *N. forsythi*. We also collected representatives
162 from 1 location on the North Island (site 7; Fig. 1A) to place genetic structure observed within
163 the Buller River catchment in a broader context. We targeted sampling sites and instream
164 microhabitat based on information from previous surveys of chironomid assemblages in New
165 Zealand in concert with our current understanding of *N. forsythi* habitat preference (Boothroyd
166 1994).

167 Ferringtonia patagonica.—We collected larvae and pupae of *F. patagonica* from 8 sites in
168 southern South America (Fig. 2). Six of these sites were in the Lakes District of Patagonian
169 Chile, and 2 were in the Neuquen Province of Argentina. We targeted sampling sites based on
170 previous surveys of Patagonian streams and according to the presence of habitat considered
171 suitable for *F. patagonica*.
172
173 *Sample collection*
174 We took samples between January 2006 and January 2010 by kick sampling with a 0.9 ×
175 0.3-mm funnel-tapered polyester sweep net and by hand removal of entire leaf packs from riffle
176 sections of each stream site. We strained bulk samples through a series of sieves to remove
177 coarse particulate organic matter and preserved all chironomid larvae in deoxyribonucleic acid
178 (DNA)-grade ethanol. We were unable to assess sample sizes of target taxa among the total
179 chironomid collection until slide preparation and high-power optical examination of individual
180 larval head capsules could be done at the Queensland University of Technology, Brisbane,

Australia. We followed the methods of Krosch et al. (2009). Logistical constraints, including inability to identify target taxa in the field and revisit sample sites, limited some sample sizes. We considered the sample sizes as best possible when interpreting patterns at such sites.

Genetic procedures

We extracted total genomic DNA from larval tissue with the Qiagen DNeasy® extraction kit (Qiagen, Hilden, Germany) and followed the manufacturer's guidelines. We amplified a 734-base pair (bp) fragment of the cytochrome *c* oxidase subunit I (COI) gene with universal invertebrate COI primers COI-s2183 (5'-CAACATTTATTTTGATTTTTTGG-3') and COI-a3014 (5'-TCCAATGCACTAATCTGCCATATTA-3') (Simon et al. 1994). We conducted polymerase chain reactions (PCR), purification, and sequencing as described by Krosch et al. (2011b). We deposited all sequences in GenBank (Accession Numbers XXXX-XXXX).

Data analyses

We aligned COI sequences and edited them by eye in BioEdit (version 7.0.5; Hall 1999). We conducted tests for sequence saturation (an indicator of potential homoplasy) by calculating the mean ratio of transitions to transversions in MEGA (version 4.0; Tamura et al. 2007). We calculated the population parameter θ_π (a diversity estimate based on the mean number of pairwise differences among populations) and gene diversity (equivalent to expected heterozygosity) in Arlequin (version 3.11; Excoffier et al. 2005) to estimate genetic diversity within sites. We conducted analysis of molecular variance (AMOVA) in Arlequin to assess partitioning of variation within and among sites. We estimated Tajima's *D* tests of neutrality for the entire data set using 1000 coalescent simulations in DnaSP (version 5.0; Librado and Rozas

2009) to determine if sequences were evolving neutrally.

We used the online resource FindModel (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html) to identify the model of nucleotide substitution that best fitted each COI data set. We used this model for all subsequent phylogenetic reconstruction wherever software options allowed. We inferred gene trees based on COI sequences with neighbor-joining inference (NJ; 10,000 bootstraps) under the General-Time Reversible (GTR) model of evolution, incorporating a gamma (G) distribution of nucleotide substitution rates and maximum parsimony (MP; 1000 bootstraps, tree bisection and reconnection [TBR] branch-swapping, 1 random addition replicate) reconstruction as implemented in PAUP* (version 4.0; Swofford 2001). We conducted Bayesian inference of phylogeny (B; 5×10^6 generations) in MrBayes (version 3.1.2; Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) under the GTR + G model of sequence evolution. Convergence of the Bayesian analysis was maximized by ensuring the standard deviation of split frequencies was <0.01 and by performing multiple runs. We did maximum likelihood (ML; 1000 bootstraps) reconstruction in RAxML (version 7.0.3; Stamatakis 2006) under the GTRMIX model of sequence evolution. For *N. forsythi*, we sequenced individuals of *Anzacladius kiwi* Cranston and *Botryocladius freemani* Cranston and Edward, along with a putative 2nd species of stream-dwelling *Naonella* (*N.* sp. n. 1) as outgroups. For *F. patagonica*, we sequenced individuals of *Botryocladius edwardsi* Cranston and Edward and *E. martini* as outgroups. We calculated mean among-lineage corrected pairwise divergences in MEGA under the Tamura–Nei (TN93) model of evolution because this model is the most complex and, thus, the most appropriate model available in MEGA.

We estimated times to most recent common ancestor (tmrca) for relevant nodes with the BEAST software package (version 1.5.3; Drummond and Rambaut 2007) under the GTR + G

model of evolution. We used Bayesian consensus topologies for each taxon as starting trees to reduce computational time. We fixed a chironomid mitochondrial divergence rate of 1.5% Ma^{-1} $lineage^{-1}$ calculated from divergence-time estimates of Nearctic and Palearctic *Chironomus* species (Martin et al. 2002) as the initial mean rate of molecular evolution under a relaxed log-normal molecular clock. We used a Speciation: Yule Process tree prior because it is considered the most appropriate prior for intraspecific estimates of tmrcas where branch lengths among divergent sympatric clades are long. We did 4 runs of 50×10^6 generations each for *N. forsythi* and 3 runs of 40×10^6 generations each for *F. patagonica*. Different run lengths between the 2 data sets reflect the difference in the number of generations required for estimates of tmrca to obtain appropriate support (e.g., effective sample sizes >200).

We tested hypotheses of post-isolation population expansion with various methods. We estimated Fu's F_S (Fu 1997) for each lineage in DnaSP. For each lineage, we plotted mismatch distributions of frequencies of pairwise differences in DnaSP. We used the raggedness metric in DnaSP to test a null hypothesis of historical population expansion. We ran 1000 coalescent simulations to assess statistical support for Fu's F_S and raggedness metrics. We estimated relative population size changes over time with Bayesian Gaussian Markov Random Field (GMRF) Skyride Plots in BEAST (Minin et al. 2008). We used the clock model, molecular rate, and substitution model described above and incorporated a time-aware prior on the smoothing of the scaled effective population size. We implemented 2 runs of 30×10^6 generations each for both species and combined log and tree files to produce GMRF Skyride Plots.

Results

Naonella forsythi

In total, 184 individuals of *N. forsythi* from 7 sites were sequenced for COI, representing 128 unique haplotypes (Table 1). Genetic diversity was high within sites (Table 2), and the ratio of transitions to transversions was high at 6.309. AMOVA suggested that 73.45% of the total variation occurred within sites and 26.55% among sites. Tajima's *D* tests of neutrality were not significant ($D = 1.3154$, $p = 0.925$), a result suggesting that the sequences had not evolved under selection.

Topologies produced by different methods of phylogeny reconstruction generally were concordant, but statistical support for nodes varied (Fig. 3). Four highly divergent mitochondrial lineages were well supported by 2 of the 3 methods. Lineage 4 was not monophyletic under ML. Support for nodes connecting lineages was generally high across methods, particularly the Bayesian reconstruction in which only 1 major node showed a posterior probability <1.00. All methods placed a putative novel species of *Naonella* (*N*. sp. n. 1) consistently as sister to all *N. forsythi*. Corrected mean pairwise divergence among lineages ranged from 5.30% between Lineages 3 and 4 to 10.1% between Lineages 1 and 2 and 1 and 3. Divergence estimates between the 4 lineages and the putative novel species were slightly higher, ranging from 12.2 to 13.4%.

Distributions of the 4 divergent lineages identified here were largely incongruent with geographical location. Each lineage included individuals from multiple streams that often were separated by large geographical distances (Fig. 1A, B). In particular, the widely distributed Lineage 1 was recovered from all 7 sampling sites, including site 7 on the North Island, whereas Lineage 3 was sampled from 4 sites on the South Island. Lineages 2 and 4 were sampled from 3 sites each. However, for both lineages, 1 site (site 1) was represented by only a single individual. In Lineage 2, the 2 remaining sites, sites 3 and 4, were geographically proximate (<15 km), whereas the remaining 2 sites in Lineage 4, sites 5 and 6, are separated by ~70 km.

Estimates of tmrca for major nodes of the inferred phylogeny suggested that diversification in *N. forsythi* began around 5.78 Ma (7.44–4.27 Ma; Table 3) just prior to the Mio–Pliocene boundary, roughly coinciding with severe cooling considered suggestive of major glaciations in the region (Mercer 1983). Diversification in *N. forsythi* apparently continued throughout the Pliocene, culminating in the radiation of haplotypes belonging to Lineage 3 around 2.32 Ma (3.48–1.35 Ma).

The Fu's F_S estimate of population expansion across the entire data set was large, negative, and significantly different from that expected under a model of constant population size (F_S = –23.902, p = 0.011). When each lineage was tested separately (Table 4), only Lineage 4 was not significantly supported as having undergone population expansion. Mismatch distributions for all 4 lineages appeared to be multimodal (Fig. S1a–d; available online from: http://dx.doi.org/10.1899/12-044.1.s1). However, we could not reject a null hypothesis of recent population expansion with tests of raggedness (Table 4). This result was further supported by a putative rise in effective population size from ~5 Ma implied by the Bayesian GMRF Skyride plot (Fig. S2a; available online from: http://dx.doi.org/10.1899/12-044.1.s2).

Southern South America: Ferringtonia patagonica

Collections at 8 sites on either side of the Andean mountain range in central Chile and Argentina recovered 62 individuals of *F. patagonica* (Table 1). I count 48 in Table 2 unique COI haplotypes were identified across the study region, and within-site genetic diversity was high (Table 2). AMOVA suggested that 12.1% of the variation was among sites, and the remaining 87.9% was within sites. The ratio of transitions to transversions was relatively high at 5.125 and Tajima's D tests for neutrality confirmed that sequence evolution had not been influenced by

296 selection (D = –0.1199, p = 0.513).

297 Four well-supported and highly divergent lineages were identified in *F. patagonica*
298 across the different methods of phylogenetic reconstruction (Fig. 4). The exception was Lineage
299 2, which was not resolved by ML. Corrected mean pairwise divergence among lineages ranged
300 from 2.00% between the close sister groups, Lineages 1 and 2, and 10.5% between Lineages 1
301 and 4 and Lineages 2 and 4. This result was reflected in the shallow divergence of Lineages 1
302 and 2 in the inferred phylogram relative to the much deeper divergence of Lineages 3 and 4.

303 No evidence was observed for ongoing bisection of discrete lineages by the Andean
304 mountain range. Sites from the western slopes did not form a monophyletic group to the
305 exclusion of sites from the east or vice versa (Fig. 2). As with *N. forsythi*, each lineage consisted
306 of individuals from multiple, often broadly distant (up to 150 km) streams. In particular,
307 Lineages 1 and 2 were distributed widely on both sides of the Andes mountain range and were
308 recovered from 8 and 7 sites, respectively. On the other hand, Lineages 3 and 4 were recovered
309 from only 2 sites each (sites 3 and 4, respectively) and were sympatric at site 2.

310 Estimated tmrcas for major nodes of the inferred phylogeny suggest that initial
311 diversification of *F. patagonica* began in the late Miocene, with all individuals of the species
312 estimated to have last shared a common ancestor ~7.44 Ma (11.10–4.41Ma; Table 3).
313 Diversification in *F. patagonica* apparently continued throughout the late Miocene and Pliocene
314 and culminated with the diversification of Lineage 3 in the early Pleistocene, 1.78 Ma (4.20–0.18
315 Ma).

316 The Fu's F_S estimate of population expansion for the total data set was negative,
317 suggesting an excess of rare alleles and, potentially, population expansion, but it was not
318 statistically significant (F_S = –6.154, p = 0.081). Likewise, Fu's F_S estimates for both Lineages 1

and 2 were large and negative when tested separately, but only Lineage 2 was significantly different. Nevertheless, mismatch distributions for those lineages that could be tested were unimodal (Fig. S1e–g), and raggedness tests supported a hypothesis of recent population expansion (Table 4). In support of this, Bayesian GMRF Skyride plots (Fig. S2b) showed a rise in effective population size from ~7.5 Ma that followed a period of steady decline.

Discussion

The goals of our study were to assess patterns of intraspecific genetic structure in New Zealand *N. forsythi* and Patagonian *F. patagonica* and to infer the effect of particular biogeographical processes on the evolution of each taxon. Our initial predictions were that the taxa would show patterns of genetic structure that reflected the individual biogeographical histories of the different study regions. Specifically, we expected *N. forsythi* to exhibit patterns of restricted gene flow among streams and *F. patagonica* to possess genetic structure congruent with Andean bisection. In contrast, patterns of genetic structure for *N .forsythi* and *F. patagonica* were strikingly similar, and both species showed patterns incongruent with our predictions.

Another goal of our study was to compare observed patterns in these taxa with our predictions of their potential dispersal, based on published patterns for the related Australian taxon, *E. martini* (Krosch et al. 2009, Krosch 2011). Like the pattern for *E. martini*, several highly divergent genetic lineages were found in both species. However, unlike the pattern for *E. martini*, lineages within *N. forsythi* and *F. patagonica* often were distributed broadly across the study regions. We argue that this pattern has been driven by historical population isolation leading to divergence of discrete mitochondrial lineages, followed by extensive post-isolation range expansion across the study areas. Differences in patterns between *N. forsythi* and *F.*

342 *patagonica* and *E. martini* appear to have been driven by variation among the study regions in
343 the availability of suitable habitat during interglacial periods.
344
345 Naonella forsythi
346 New Zealand's South Island has experienced significant tectonic upheaval and glaciation
347 since the late Miocene (Walcott 1998), including several glacial cycles that are thought to have
348 affected the study region during the Plio–Pleistocene (Suggate 1990, Suggate and Almond 2005).
349 These events caused retraction of many taxa into small isolated refugia and drove genetic
350 divergence of fragmented populations (Heads 1998, Trewick and Wallis 2001, Greaves et al.
351 2008, Wallis and Trewick 2009, Pons et al. 2011). Our data suggest that divergence of *N.*
352 *forsythi* lineages began in the late Miocene and coincided with the onset of a period of major
353 geological and climatic instability. Distributions of the highly divergent lineages show little
354 evidence for geographical endemicity within Buller River catchment. One explanation may be
355 that isolation of *N. forsythi* in multiple refugia during these periods led to divergence of observed
356 lineages, and re-expansion of some lineages during milder interglacial periods. Potentially, the
357 discrete lineages identified here arose in isolation in separate parts of the Buller River catchment
358 that remained comparatively stable at times when the main river channel became unsuitable as
359 habitat. Under this hypothesis, lineages could have recolonized previously unsuitable habitat and
360 expanded their distributions, resulting in multiple lineages that now coexist.
361 Alternatively, lineages may have colonized the Buller River catchment from neighboring
362 river systems, either through active flight or more passively via river-capture events (Waters and
363 Wallis 2000). The montane areas of the NSIP have experienced significant erosion, some of
364 which was glacier-driven, since original uplift. This erosion provided potential for dramatic

changes to catchment boundaries over time and reflected the general dynamism and unpredictability of New Zealand streams (Winterbourne et al. 1981). Population genetic data specific to the Buller River drainage are limited, but patterns of high intraspecific diversity and sympatry of divergent haplotypes have been revealed for freshwater taxa elsewhere New Zealand (see Wallis and Trewick 2009).

Ferringtonia patagonica

Southern South America, like the South Island of New Zealand, has experienced significant tectonic uplift and extensive glaciation since the late Oligocene that have altered regional geomorphology, climate, and habitat conditions dramatically (Lagabrielle et al. 2004, Blisniuk et al. 2005, Rabassa 2008). These events have led to population isolation resulting in significant genetic differentiation among populations of many taxa native to the region (Palma et al. 2002, Marchelli and Gallo 2006, Ruzzante et al. 2006, Himes et al. 2008, Ramirez et al. 2008, Victoriano et al. 2008, Sabando et al. 2011). Patterns for *F. patagonica* are broadly congruent with those recovered for *N. forsythi* in New Zealand, with divergence of *F. patagonica* lineages coincident with the proposed onset of major glaciation across the region (Mercer 1983, Rabassa and Clapperton 1990, Ehlers and Gibbard 2007, Rabassa et al. 2011) and predating the most recent and significant orogenic phase of Andean uplift (~3.5 Ma; Lagabrielle et al. 2004). Two of the 4 lineages we recovered were widespread across the study region and present on both sides of the Andes. This result implies that Andean orogeny is unlikely to have driven diversification of the observed lineages. Instead, populations of *F. patagonica* may have been isolated in multiple refugia during periods of glaciation. The sympatric distribution of certain lineages across the study region probably represents extensive postglacial range expansions. Moreover, this pattern

388 of strong genetic structure within a taxon associated with historical glaciations is consistent with
389 existing evidence from other taxa in southern South America. Patterns of genetic structure in
390 some plant and animal taxa have shown evidence for isolation in refugia that does not
391 correspond with Andean bisection (e.g., herbaceous plants, Close et al. 1978; conifers, Allnutt et
392 al. 1999; opossums, Palma et al. 2002; asters, Muellner et al. 2005).
393
394 *Potential vs realized dispersal in* N. forsythia *and* F. patagonica
395 Overall, patterns of genetic structure resolved here for both *N. forsythi* and *F. patagonica*
396 were incongruent with predictions based on their potential dispersal, as inferred from published
397 patterns for their close relative *E. martini* from eastern Australian (Krosch et al. 2009, Krosch
398 2011). Nevertheless, levels of intraspecific genetic divergence were high for all 3 taxa. This
399 divergence was correlated in *E. martini* with high geographical endemism, interpreted as the
400 result of historical isolation in rainforest refugia and with limited range expansion post-isolation.
401 In contrast, lineages within both *N. forsythi* and *F. patagonica* often were distributed widely with
402 little geographical localization over spatial scales much broader than those at which *E. martini*
403 shows endemism. Thus, *N. forsythi* and *F. patagonica* appeared to have been influenced by
404 historical population fragmentation similarly to *E. martini*, but their realized dispersal
405 subsequently differed markedly. Quite possibly, this difference simply reflects differences in
406 actual dispersal capabilities among the 3 species, i.e., *N. forsythi* and *F. patagonica* may simply
407 fly further than *E. martini*. However, all 3 taxa apparently show highly constrained habitat
408 preferences directly related to presence of riparian vegetation. Thus, it is reasonable to assume
409 that *N. forsythi* and *F. patagonica* would share with *E. martini* a low potential for dispersal
410 across unsuitable habitat. Therefore, the disparity in genetic structure may be best explained by

differences in the rate and form of recovery of streams with appropriate riparian vegetation across the 3 land masses in the period since fragmentation drove initial diversification within each taxon.

The Australian continent has undergone systemic and continued aridification that was initiated by northward drift of the continent following rifting from Antarctica in the Miocene and was exacerbated by Plio–Pleistocene glacial cycles (Martin 1998, 2006). The pollen record shows a dramatic and continued shift in historical vegetation composition across the distributional range of *E. martini*, from favorable wet closed forest to dry open sclerophyll woodland and grassland, which began in the late Miocene and resulted in a highly fragmented mosaic of habitat suitable for *E. martini* (Truswell 1993, Kershaw 1994, Martin 2006). Furthermore, the availability of permanent, flowing streams probably has declined over time in many areas because of ongoing aridification, and this decline could have further restricted movement of *E. martini* and driven genetic divergence among isolated populations. Thus, the recolonization and expansion of habitat suitable for *E. martini* probably has been extremely limited across much of the east coast resulting in a historically highly fragmented distribution. Sympatry among *E. martini* lineages was observed only in areas of postulated rain forest re-expansion between geographically proximate (<20 km apart) refugia (Nix and Switzer 1991, Hopkins et al. 1996), reflecting the general geographical endemism exhibited by this taxon (Krosch et al. 2009, Krosch 2011).

In contrast, no evidence exists for such a dramatic conversion of riparian vegetation cover and content in the Buller River drainage of New Zealand or southern South America over the same time frame (Mildenhall 1980, Arroyo et al. 1996). Thus, the floral compositions and habitat complexity of the study regions may have changed little. Distribution patterns of closed forest in

these regions during the Plio–Pleistocene were characterised by repeated cycles of contraction into fragmented refugia during glaciation events followed by expansion from refugia during interglacial periods (Freeman 1959, McGlone 1985, Leschen et al. 2008, Iglesias et al. 2011). Furthermore, the availability of permanent streams in these regions is unlikely to have changed greatly in postglacial periods. Thus, distributions of plant taxa were greatly reduced and highly fragmented during glacial periods in New Zealand and South America (promoting divergence among isolated populations), but expansion of habitat suitable for *N. forsythi* and *F. patagonica* during interglacial periods may have facilitated dispersal and recontact of divergent lineages. Observed widespread geographical distributions of some lineages of *N. forsythi* (≥80 km) and *F. patagonica* (≥150 km) imply that realized dispersal has been more extensive across the study regions in these taxa than in *E. martini*. Moreover, although this result suggests that these taxa possess some capacity for dispersal among sites, dispersal appears to have been patchy and restricted by the availability of suitable intervening habitat. Taken together, these results suggest that orthoclad chironomids may be particularly susceptible to reduction and fragmentation of suitable habitat, a pattern mirrored by many montane aquatic invertebrates globally (Taylor et al. 1998, Witt and Hebert 2000, Hogg et al. 2002, Miller et al. 2002, Monaghan et al. 2002, Bunje 2005, Pauls et al. 2006, Smith et al. 2006a, Kubow et al. 2010).

An alternative explanation for the current pattern is that observed divergent sympatric lineages represent the result of sympatric speciation. In chironomids, this process is most likely to occur through asynchronous emergence of adults from pupae, resulting in multiple breeding cohorts present in a single stream (‘patchy recruitment;’ Bunn and Hughes 1997). However, such asynchronous adult emergence is uncharacteristic of temperate ecosystems and no evidence for this phenomenon has been found in *N. forsythi* or *F. patagonica*. Furthermore, the patterns

457 resolved here derive from a single, maternally inherited genetic locus. Thus, hypotheses of
458 sympatric speciation must await proper assessment using, for example, appropriate nuclear loci,
459 to investigate the possibility that observed lineages may represent biological species. Were such
460 markers to exhibit similar patterns of structure to those presented here, some support would exist
461 for hypotheses that observed divergences are species-level differences. In contrast, were nuclear
462 data to differ from patterns resolved here, support would exist for sex-biased dispersal or other
463 scenarios having been important in the evolution of these 2 taxa.

464 In conclusion, few investigators to date have compared patterns of genetic structure
465 among closely related taxa that are distributed on different continents (but see Albach et al. 2006,
466 Pons et al. 2011, Cook et al. 2012). We think this approach can allow inference of common taxic
467 responses to historical biogeographical events. Our study is the first detailed assessment of
468 genetic structure in any New Zealand or Neotropical chironomid species and is one of very few
469 concerning freshwater invertebrates on the South Island of New Zealand or in southern South
470 America. Moreover, our study contributes to our understanding of the recolonization dynamics
471 of freshwater taxa following putative glacier-driven population fragmentation. Future
472 investigations would benefit from more comprehensive sampling of each taxon's distribution to
473 place currently understood diversity in a broader regional context. In *N. forsythia*, sampling is
474 needed from adjacent catchments on the western side of the Southern Alps, locations on the
475 eastern side of the Southern Alps, and additional sites from the North Island of New Zealand.
476 Additional sites in southern South America should be sampled for *F. patagonica*, particularly on
477 the eastern side of the Andes.
478

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763 **Figure Captions**

764 Fig. 1. Geographical locations of *Naonella forsythi* sampling sites in North Island (A) and South
765 Island (B), New Zealand. Site numbers are: 1) Borlase Creek, 2) Speargrass Creek, 3)
766 Hope River, 4) Owen River, 5) unnamed creek near Lake Rotoroa (Rotoroa Creek), 6)
767 Jackson Creek, 7) unnamed creek near Ohakune (Ohakune Creek). Geographical
768 distributions of cytochrome *c* oxidase subunit I (COI) lineages are represented by solid or
769 dashed lines. Thick solid lines in the inset and panel B represent the approximate location
770 of the Southern Alps ridgeline.

771 Fig. 2. Geographical locations of *Ferringtonia patagonica* sampling sites in southern South
772 America. Site numbers are: 1) Rio Palguin, 2) Rio Don Baucha, 3) Estero La Casilla, 4)
773 Estero Correntoso, 5) Rio Chaleufu, 6) unnamed creek near Peulla (Peulla Creek), 7)
774 Arroyo Quilanlahue, 8) Arroyo Partido. Geographical distributions of cytochrome *c*
775 oxidase subunit I (COI) lineages are represented by solid or dashed lines. Long dashed
776 line running north–south represents the political boundary of Chile and Argentina and the
777 Andean ridgeline.

778 Fig. 3. Consensus Bayesian topology for *Naonella forsythi* cytochrome *c* oxidase subunit I (COI)
779 sequences with branch lengths representing expected substitutions per site. Node-
780 associated support values for major nodes are as follows: above branch = Bayesian
781 posterior probability (PP)/maximum parsimony (MP) bootstrap probability, below branch
782 = maximum likelihood (ML) bootstrap/neighbour-joining (NJ) bootstrap probabilities; *
783 indicates PP = 1.00 or MP/NJ bootstrap probabilities = 100, NR indicates a node that was
784 not resolved. Lettered nodes are those for which time to most recent common ancestor
785 (tmrca) was estimated (Table 2).

Fig. 4. Consensus Bayesian topology for *Ferringtonia patagonica* COI sequences with branch lengths representing expected substitutions per site. Node-associated values for relevant nodes are as follows: above branch = Bayesian posterior probability (PP)/maximum parsimony bootstrap probability (MP), below branch = maximum likelihood (ML) bootstrap/neighbor-joining bootstrap probabilities; * indicates PP = 1.00 or MP/ML = 100, NR indicates a node that was not resolved. Lettered nodes are those for which time to most recent common ancestor (tmrca) was estimated (Table 2).

795 Table 1. Geographical locations of New Zealand and South American sampling sites and sample
796 sizes at each site. Ck = creek, CH = Chile, ARG = Argentina

Site name (country of origin)	Site number	Latitude	Longitude	Elevation (m asl)	Sample size
New Zealand					
Borlase Ck	1	41°48.24'S	172°50.45'E	678	32
Speargrass Ck	2	41°46.50'S	172°46.30'E	562	27
Hope River	3	41°41.38'S	172°37.05'E	360	41
Owen River	4	41°41.02'S	172°27.14'E	245	25
Rotoroa Ck	5	41°48.11'S	172°32.31'E	675	30
Jackson Ck	6	42°22.17'S	172°16.01'E	500	27
Ohakune Ck	7	39°24.20'S	175°27.15'E	578	2
South America					
Rio Palguin (CH)	1	39°27'S	71°48'W	825	2
Rio Don Baucha (CH)	2	39°23'S	71°47'W	680	13
Estero La Casilla (CH)	3	39°14'S	71°56'W	240	4
Estero Correntoso (CH)	4	39°18'S	72°04'W	215	11
Rio Chaleufu (CH)	5	40°44'S	72°18'W	465	1
Peulla (CH)	6	41°05'S	72°01'W	270	3
Arroyo Quilanlahue (ARG)	7	40°09'S	71°33'W	660	13
Arroyo Partido (ARG)	8	40°14'S	71°22'W	1185	15

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Table 2. Summary statistics for *Naonella forsythi* and *Ferringtonia patagonica*. θ_π is a population-level diversity estimate based on the mean number of pairwise differences among populations.

Site number	Sample size	Number of haplotypes	Mean gene diversity ± SE	θ_π
New Zealand				
1	32	19	0.901 ± 0.046	38.71
2	27	22	0.983 ± 0.015	41.35
3	41	37	0.995 ± 0.006	31.04
4	25	20	0.980 ± 0.018	46.49
5	30	24	0.963 ± 0.026	30.09
6	27	14	0.895 ± 0.039	43.33
7	2	2	1.000 ± 0.500	15.00
South America				
1	2	2	1.000 ± 0.500	21.00
2	13	13	1.000 ± 0.030	43.32
3	4	4	1.000 ± 0.177	47.50
4	11	7	0.873 ± 0.090	16.84
5	1	1	1.000 ± 0.000	N/A
6	3	2	0.667 ± 0.310	12.00
7	13	11	0.950 ± 0.040	11.98
8	15	8	0.900 ± 0.070	9.240

804 Table 3. Times to most recent common ancestor (tmrca) estimated for relevant nodes for the
805 *Naonella forsythi*, *Naonella* sp. n. 1, *Ferringtonia patagonica*. Node labels follow Figs 3 and 4
806 for *N. forsythi* and *F. patagonica*, respectively. Ma = 10^6 y ago.

Node label	Lineage(s) included	tmrca (Ma)	95% credibility intervals (Ma)	Effective sample size
N. forsythi				
A	*N. forsythi* and *N.* sp. n. 1	6.31	8.25–4.59	420.41
B	All ingroup	5.78	7.44–4.27	355.37
C	2 + 3 + 4	4.71	6.15–3.28	229.80
D	1	4.00	5.46–2.70	371.39
E	3 + 4	3.41	4.74–2.21	249.88
F	2	2.99	4.37–1.77	233.50
G	4	2.58	3.85–1.44	714.92
H	3	2.32	3.48–1.35	242.81
F. patagonica				
A	All ingroup	7.44	11.10–4.41	412.52
B	1 + 2 + 3	6.02	9.20–3.37	350.34
C	1 + 2	4.54	7.41–2.30	231.55
D	1	3.47	5.91–1.56	267.10
E	2	3.45	5.92–1.48	305.97
F	4	2.15	4.32–0.49	596.40
G	3	1.78	4.20–0.18	1339.41

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Table 4. Estimates of Fu's F_S and raggedness for each lineage of *Naonella forsythi* and *Ferringtonia patagonica*. θ_π is a population-level diversity estimate based on the mean number of pairwise differences among populations. Values in bold are statistically significant ($p < 0.05$).

Lineage number	Sample size	θ_π	Fu's F_S	*p*-value	Raggedness	*p*-value
N. forsythi						
1	107	13.94	–24.14	**0.000**	0.0183	0.802
2	21	13.56	–10.86	**0.000**	0.0134	0.141
3	36	5.32	–7.017	**0.007**	0.1759	0.985
4	20	9.82	1.411	0.746	0.1253	0.886
F. patagonica						
1	30	7.782	–3.603	0.100	0.2179	0.153
2	24	6.413	–4.526	**0.036**	0.3517	0.641
3	3	4.000	0.133	0.249	–	–
4	4	8.500	0.265	0.328	0.5278	0.492